UNIVERSITI PUTRA MALAYSIA

PATHOLOGICAL, BACTERIOLOGICAL AND PREVALENCE STUDIES OF OVINE FOOTROT

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By

KARIM ALWAN MOHAMED Al-JASHAMY

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of Requirements for the Degree of Doctor of Philosophy

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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

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April 2003

Chairman: Associate Professor Dr. Jasni Sabri, DAHP, DVM, PhD
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Ovine footrot, is a disease associated with infection by the bacterium *Dichelobacter nodosus*. It is a disease that limits the productivity of sheep-farming enterprises throughout the world. Both wool production and body weight are adversely affected during the clinical phase of the infection.

Ovine footrot has become an important contagious disease in Malaysia. The first confirmed case of footrot was reported in a government sheep farm in mid-1980s. The disease is now present in other farms throughout the country, and local vaccine is being used to reduce the disease.

Previous studies have identified *D. nodosus* in three sheep farms in Malaysia and only serogroup B was identified. The possible presence of other *D. nodosus* serogroups and serotypes is unknown. This study attempts to isolate

and identify the unknown serogroups and serotypes so as develop a better vaccine candidate using local isolates of *D. nodosus*.

Eight sheep farms were investigated in this study. Four sheep farms were found to be infected with *D. nodosus.* Two hundred and ninety-three *D. nodosus* isolates were obtained from 741 foot samples. Five serogroups were identified in Malaysia. This is the first study where serogroups A, C, F and I with their serotypes A1, A2, C1, F1 and F2 were identified in the infected sheep farms. Serogroup B was the predominant serogroup isolated (78.2%) while the isolation percentages for serogroups F, A, I and C were 7.9%, 7.5%, 3.8% and 2.7% respectively.

The information on the pathogenesis of the disease is still lacking despite previous studies on ovine footrot. Interdigital cutaneous changes associate with footrot in sheep is not well documented. The disease was induced experimentally in sheep by topical application of bacterial isolates on the interdigital skin of the hoof, and light and electron microscopy studies of the lesions were conducted.

Virulent footrot was observed by a gross progressive separation of the horny tissues from the soft tissues. On day 21 post inoculation (p.i.), a complete separation of the hoof from the underrunning structures and lameness were evident. The benign footrot was observed with mild interdigital dermatitis and all infected feet completely recovered on day 21 p.i..

Histopathological changes in virulent footrot were observed in the interdigital skin layers and hoof matrix. These ranged from acute dermatitis to hyperkeratosis, parakeratosis and acanthosis of the epidermis. Oedema and leukocytic infiltration with neutrophils, macrophages and scanty lymphocytes were also evident in the dermis. Furthermore, vasculitis and perivascular cuffing, lymphangitis and inflammation of the sweat glands were observed in the dermis. The histopathological changes of benign footrot were less severe than virulent form in the epidermis and there were no pathological changes in the dermis.

In scanning electron microscopy, a severe zone of lysis appearing as a surface depression around bacteria in the horny layer of the interdigital skin of the hoof was detected in virulent footrot, while this lesion was less severe in the benign form. Transmission electron microscopy revealed degeneration in the epidermis and dermis. Degeneration in the basal cell layer of the epidermis and the basement membrane in virulent form of footrot, which have not been reported previously was observed in this study.

Dichelobacter nodosus was observed in the lesions of the epidermis and dermis of virulent footrot. Its' isolation from characteristic foot lesions indicated that it was associated with footrot. Immunohistochemistry observations validate the relationship between the lesions seen in footrot and virulent *D. nodosus*. Immunogold staining technique facilitates to detection and localisation of *D. nodosus* for electron microscopy. Specific reactions were labelled in

components and the matrix of epidermis and dermis of the interdigital skin. *Dichelobacter nodosus* antigen labelled with 5 nm gold particles was observed in the intracellular and intercellular spaces of the epidermis. This is the first report where immunogold labelling technique have been used in the study of footrot lesions in sheep for electron microscopical observations.

The total monthly rainfall and mean daily temperature have a relation to the prevalence rate of the disease. These conditions provide suitable environment propagation of *D. nodosus*. The overall prevalence of footrot in the eight farms investigated was 3.3%. The highest prevalence was recorded in April (0.8 %), while the lowest in August (0.3%) in IHK farm by survey study. Observations described in this study were made to define the prevalence are related to seasonal conditions, but the effect of rainfall overrides all other factors for footrot to occur.

Adults were more susceptible than weaners. No cases were detected in preweaners. The prevalence by sex which was 4.4% in the male and 7.7% in the female was significant ($p=0.009$). No significant difference in prevalence rates between breeds was detected.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra
Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KAJIAN PATOLOGI, BAKTERIOLOGI DAN PREVELANS BURUK KAKI OVIN

Oleh

KARIM ALWAN MOHAMED AL-JASHAMY

April 2003

Pengerusi: Profesor Madya Dr. Jasni Sabri, DAHP, DVM, PhD
Fakulti: Perubatan Veterinar

Buruk kaki adalah penyakit yang disebabkan olah bakteria *Dichelobacter nodosus*. Penyakit ini mengurangkan produksi bebiri di seluruh dunia. Berat badan dan pengeluaran bulu bebiri terjejas akibat jangkitan bakteria ini.

Buruk kaki telah menjadi penyakit berjangkit yang penting di Malaysia. Kes pertama buruk kaki pada bebiri pernah dilaporkan berlaku di sebuah ladang ternakan bebiri kerajaan pada pertengahan tahun 1980. Sekarang penyakit ini sudahpun dilaporkan di ladang ternakan bebiri di negari lain di Malaysia dan vaksin tempatan digunakan untuk mengurangkan kejadian penyakit ini.

Kajian yang lepas telah mengesan *D. nodosus* di tiga ladang bebiri di Malaysia dan hanya serogroup B sahaja yang dapat dikesan. Samada terdapat serogroup dan serotip lain di sini tidak ditemui lagi. Kajian ini dijalankan untuk mengesan serogroup dan serotip yang tidak di ketahui sebelum ini untuk membolehkan penghasilan vaksin yang lebih baik dengan menggunakan isolat tempatan. Kajian

telah dilakukan di lapan ladang ternakan bebiri. Dua ratus dan sembilan puluh tiga isolat *D. nodosus* telah berjaya dipencilkan dari 741 sampel kaki. Lima serogroup telah dapat dikesan. Buat pertama kalinya serogroup A, C, F, dan I dengan serotip A1, A2, C1, F1 dan F2 telah dikesan di ladang bebiri yang telah dijangkiti. Serogroup B adalah serogroup yang paling banyak di isolat (78.2%) dan peratusan isolat untuk masing-masing serogroup F, A, I dan C adalah 7.9%, 7.5%, 3.8% dan 2.7%.

Pengetahuan tentang patogenesis penyakit ini masih lagi kabur walaupun banyak kajian telah dilakukan sebelum ini. Tidak banyak laporan tentang perubahan interdigital kutaneous yang berlaku semasa buruk kaki. Penyakit buruk kaki virulen telah dihasilkan dengan menyapu isolat bakteria pada kulit interdigital pada kuku keras dan lesi dikaji dengan menggunakan mikroskop cahaya dan elektron. Buruk kaki virulen dilihat sebagai berlakunya pemisahan yang progresif tisu keras daripada tisu lembut kaki. Pada hari ke 21 selepas disuntik, pemisahan lengkap kuku keras daripada struktur bawahan menyebabkan ketempangan berlaku. Dalam buruk kaki benigna, dermatitis interdigital yang tidak teruk berlaku dan semua kaki sembuh dengan sempurna pada hari ke 21 selepas suntikan bakteria. Perubahan histopatologi dalam buruk kaki virulen dapat dilihat pada lapisan kulit interdigital dan matrik kuku keras. Lesi yang berlaku adalah dari dermatitis akut ke hiperkeratosis, parakeratosis dan akantosis di epidermis. Edema dan penyusupan neutrofil, makrofaj and sedikit limfosit juga kelihatan di dermis. Selain daripada itu, terjadi vaskulitis dan "cuffing" perivaskular, limfangitis dan inflamasi kelenjar peluh di dermis.

Perubahan histopatologi buruk kaki benigna adalah kurang teruk daripada buruk kaki virulen di epidermis dan tiada perubahan patologi berlaku di dermis. Melalui mikroskopi elektron imbasan, satu zon lisis yang teruk yang mempunyai satu lekukan di sekeliling bakteria pada lapisan kuku keras interdigital kulit kelihatan dalam buruk kuku virulen. Lesi ini kurang teruk dalam buruk kuku benigna. Melalui mikroskopi elektron transmisi, degenerasi dilihat di epidermis dan dermis. Degenerasi sel basal epidermis dan selaput basemen buruk kaki virulen yang tidak pernah dilaporkan sebelum ini juga di temui dalam kajian ini.

Dichelobacter nodosus dapat dilihat di epidermis dan dermis buruk kaki virulen. Pemencilan bakteria ini daripada lesi buruk kaki menunjukkan bahawa lesi ini berkaitan dengan kehadiran bakteria ini.

Pemeriksaan secara imunohistokimia menyokong yang kejadian lesi buruk kaki berkaitan dengan kehadiran dan virulen *D. nodosus*. Pewarnaan "immunogold" telah digunakan untuk mengesan dan mencari lokasi *D. nodosus* menggunakan mikroskop cahaya dan elektron. Satu reaksi yang spesific dilabel di komponen intrasel dan matriks epidermis dan dermis kulit interdigital.

Antigen *D. nodosus* yang dilabel dengan 5 nm zarah emas dilihat dalam ruang intersel dan intrasel epidermis. Reaksi pewarnaan imuno lesi buruk kaki benigna adalah kurang berbanding buruk kaki virulen di lapisan kulit interdigital. Teknik

perlabelan "immunogold" ini adalah pertama kali digunakan untuk mengkaji buruk kaki pada bebiri dengan menggunakan mikroscop cahaya dan elektron.

Jumlah taburan hujan bulanan dan min suhu harian ada kaitan dengan prevalen penyakit ini. Keadaan ini menyediakan persekiatran yang sesuai untuk pembiakan *D. nodosus.* Prevalen penyakit buruk kaki di lapan ladang yang dikaji keseluruhanmya adalah 3.3%.

Prevalen yang paling tinggi telah direkod pada bulan April (0.8%) dan yang terendah pada bulan Ogos (0.3%) di ladang IHK secara "survey". Kajian ini menunjukkan bahawa prevalen berkaitan dengan musim, tetapi hujan yang berlaku melebihi faktor yang lain dalam menyebabkan kejadian buruk kaki. Umur adalah sangat bererti pada bebiri dewasa berbanding bebiri yang sudah di cerai susu. Prevalen yang mengikut jantina adalah 4.4% pada bebiri jantan dan 7.7% pada bebiri betina adalah bererti ($p=0.009$). Prevalen mengikut baka didapati tidak bererti.

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I certify that an Examination Committee met On 26th April 2003 to conduct the final examination of Karim Alwan Mohamed Al-Jashamy on his Philosophy Degree of Science thesis entitled "Pathological, Bacteriological and Prevalence Studies of Ovine Footrot" in according with Universiti Pertanian Malaysia **(Higher Degree) act 1980 and Universiti Putra Malaysia (Higher Degree)** regulation 1981. The Committee recommended that the candidate be awarded the relevant degree. Members of the examination committee are a follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follow:

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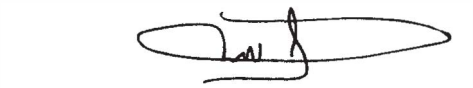
Date: 12 SEP 2003

I hereby declare that the thesis is based on my original work except for quotations and citation, which have been duly acknowledged. I also declare that it has been not been previously or concurrently submitted for any other degree at Universit Putra Malaysia or other institutions.



Karim Alwan Mohamed Al-Jashamy
Date: 10 July, 2003

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